5 ACID/BASE/NEUTRAL DRUG SCREEN AND QUANTATION BY GC-NPD AND/OR GCMS

5.1 Summary

5.1.1 Acidic, basic and neutral drugs are extracted from biological fluids or tissues using solid phase extraction (SPE) or liquid-liquid extraction (LLE) followed by instrumental analysis with gas chromatography-nitrogen-phosphorus detection (GC-NPD) and/or gas chromatography-mass spectrometry (GCMS). This procedure may employ several drug mixes, but at least one positive control and one negative control. The procedure may be used to screen for basic, acidic and neutral drugs. Once drugs have been confirmed, the procedure may be used to quantitate drugs provided at least 3 calibrators are used to generate a response curve.

5.2 Specimen Requirements

5.2.1 1-3 mL whole blood, urine, bile, gastric contents, other fluids or tissue homogenates.

5.3 Reagents And Standards

5.3.1 Ammonium hydroxide

5.3.2 Glacial Acetic Acid

5.3.3 Potassium Hydroxide

5.3.4 Potassium Phosphate

5.3.5 Ethyl Acetate

5.3.6 Methanol

5.3.7 Acetonitrile

5.3.8 Dichloromethane

5.3.9 Isopropyl alcohol

5.3.10 Hexane

5.3.11 Toluene

5.3.12 Isoamyl alcohol

5.3.13 Monobasic Potassium Phosphate/Disodium Phosphate Buffer Concentrate (Fisher)

5.3.14 Sodium phosphate (NaH_2PO_4)

5.3.15 Chloroform

5.3.16 Hydrochloric Acid

5.3.17 Sodium tetraborate decahydrate

5.3.18 Sodium hydrogen carbonate

5.3.19 Potassium carbonate

5.4 Solutions, Internal Standards, Calibrators and Controls

5.4.1 Solutions for Varian SPE Extraction

5.4.1.1 1 M Acetic Acid Add 5.75 mL of glacial Acetic Acid to a 100 mL volumetric flask half filled with dH_2O. QS to volume with dH_2O.

5.4.1.2 5.0 M Potassium Hydroxide Weigh 28 g of potassium hydroxide into a 100 mL beaker containing approximately 70 mL dH_2O. After the potassium hydroxide has dissolved, transfer to 100 mL volumetric flask and QS to volume with dH_2O.

5.4.1.3 0.1 M Phosphate Buffer, pH 6.0 Weigh out 13.61 g of KH_2PO_4 and transfer into a 1 L volumetric flask containing approximately 800 mL of dH_2O. Adjust the pH of the above solution to 6.0 by the addition of 5.0 M potassium hydroxide while stirring. QS to volume with dH_2O.

5.4.1.4 2 % Ammonium Hydroxide in Ethyl Acetate Pipette 2 mL of concentrated ammonium hydroxide into a 100 mL graduated cylinder filled with 98 mL of ethyl acetate. Cap graduated cylinder with a glass stopper, and mix well. Vent cylinder occasionally. PREPARE SOLUTION FRESH DAILY!

5.4.2 Solutions for UCT CleanScreen® SPE Extraction

5.4.2.1 When using UCT CleanScreen® SPE Extraction columns, either sodium or potassium phosphate buffer may be used. However, the same buffer (sodium or phosphate) must be used throughout the duration of the procedure.

5.4.2.2 0.1 M Potassium Phosphate Buffer, pH 6.0. Weigh out 13.61 g of KH_2PO_4 and transfer into a 1 L volumetric flask containing approximately 800 mL of dH_2O. Adjust the pH of the above solution to 6.0 by the addition of 5 M potassium hydroxide while stirring. QS to volume with dH_2O. Solution can also be purchased (e.g. Fisher).

OR

5.4.2.3 0.1 M Sodium Phosphate Buffer, pH 6.0. Weigh out 1.70g Na_2HPO_4 and 12.14g $NaH_2PO_4 \cdot H_2O$ and transfer to a 1 L volumetric flask containing approximately 800 mL dH_2O. Adjust the pH of the above solution to 6.0 by the addition of 5 M sodium hydroxide. QS to volume with dH_2O. Solution can also be purchased (e.g. Fisher).

5.4.2.4 1.0 M Acetic Acid. Add 5.75 mL of glacial Acetic Acid to a 100 mL volumetric flask half filled with dH_2O. QS to volume with dH_2O.

5.4.2.5 Ethyl acetate/Hexane, 50:50 v/v. Mix 500 mL ethyl acetate with 500 mL hexane.

5.4.2.6 Dichloromethane/isopropanol 80:20 v/v. Mix 800 mL dichloromethane with 200 mL isopropanol.

5.4.2.7 Dichloromethane/isopropranol/ammonium hydroxide elution solvent. Add 2 mL concentrated ammonium hydroxide to 100 mL dichloromethane/isopropanol 78:20 v/v. PREPARE SOLUTION FRESH DAILY!

5.4.3 Solutions for liquid/liquid base extaction

5.4.3.1 Saturated borate buffer solution. Add sodium tetraborate decahydrate to dH_2O until no more dissolves after shaking vigorously. Decant saturated solution into a glass jar equipped with a volumetric dispenser.

5.4.3.2 Toluene:Hexane:Isoamyl Alcohol (THIA) extraction solvent (78:20:2), v:v:v: Mix toluene (780 mL), hexane (200 mL), and isoamyl alcohol (20 mL).

5.4.3.3 Sodium Hydrogen Carbonate/Potassium Carbonate (dry 3:2 w/w) Mix 300 g $NaHCO_3$ with 200 g K_2CO_3.

5.4.4 Reagents for liquid/liquid acid/neutral extraction

5.4.4.1 1.0 M sodium phosphate buffer (pH 5.5): Weight 13.8 g sodium phosphate into a 100 mL volumetric flask and QS to volume with dH_2O. Adjust pH to 5.5 with 5 M ammonium hydroxide.

5.4.4.2 0.1 N HCl. Pipet 8.3 mL concentrated hydrochloric acid into a 1 L volumetric flask and QS to volume with dH_2O.

5.4.5 Internal Standard

5.4.5.1 Prepare internal standards from 1 mg/mL drug standards. The concentration of the internal standard should be approximately midrange of suspected analytes. Suitable internal standards included Sertis, methapyrilene, mepivicaine, phensuximide, hexobarbital or deuterated standards (if analyzed by GCMS in SIM mode). The concentration of internal standard will vary depending on type of case analyzed (DUID vs postmortem).

5.4.6 Calibrators

5.4.6.1 For quantitative analyses, a minimum of 3 different calibrators must be used for each analyte. The concentration of the calibrators must bracket the concentration in the unknown case specimens. If the concentration of the specimen exceeds the concentration of the highest calibrator, the specimen should be diluted and re-extracted for accurate quantitation. Otherwise the specimen should be reported as having a concentration greater than the highest calibrator. If the concentration of the specimen falls below the lowest calibrator, then the specimen can be reported as containing the analyte at less than the lowest calibrator.

5.4.7 Controls

5.4.7.1 Positive controls are prepared to monitor the performance of the assay. These controls may vary depending on type of case (DUID vs postmortem). The positive control should contain frequently observed drugs at concentrations similar to the lower reporting limit to address sensitivity of the assay. In addition, the positive control should contain drugs of various chromatographic retention times (early and late eluting drugs) to ensure the chromatographic conditions are capable of detecting a number of drugs. The positive controls can be prepared in house or purchased from Quality Assurance Systems (QAS).

5.4.7.2 For quantitative procedures the controls must be prepared from a different manufacturer or lot number than the calibrators. When this is not practical or possible, the control should at least be prepared using a different weighing of the material used for the calibrators or prepared by a different analyst. Control concentration must be between lowest and highest calibrator (approximately midrange).

5.4.7.3 Negative Control. Blood bank blood previously determined not to contain reportable drugs (i.e. most bloods contain nicotine and caffeine but these drugs are not typically reported).

5.5 Apparatus

5.5.1 Agilent GC/MSD and/or GC/NPD, Chemstation software, compatible computer & printer

5.5.2 Test tubes, 16 x 125 mm round bottom, screw cap tubes, borosilicate glass with Teflon caps

5.5.3 Test tubes, 16 x 125 mm round bottom tubes, borosilicate glass

5.5.4 Test tubes, 16 x 114 mm (10 mL) glass centrifuge, conicals

5.5.5 Test tubes, 13 x 100 mm round bottom, screw cap tubes, borosilicate glass

5.5.6 Centrifuge capable of 2,000 – 3,000 rpm

5.5.7 Varian Bond Elute LRC Certify solid phase extraction (SPE) columns or Cleanscreen® Extraction Cartridges (ZSDAU020) from United Chemical Technologies (200 mg columns)

5.5.8 Solid phase extraction manifold

5.5.9 Vortex mixer

5.5.10 Evaporator/concentrator

5.5.11 GC autosampler vials and inserts

5.5.12 Test tube rotator

5.5.13 GC NPD parameters. Instrument conditions may be changed to permit improved performance.

5.5.13.1 Oven program.

- Equilibration time: 0.50 minutes
- Initial temp: 110 °C
- Initial time: 1 minutes
- Ramp: 10 °C/min
- Final Temp: 290 °C
- Final Time: 10 minutes
- Run Time: 29 minutes

5.5.13.2 Inlet.

- Mode: Splitless
- Temperature: 270 °C
- Constant pressure: 30 psi
- Purge flow: 60 mL/min
- Purge time: 0.75 min
- Total flow: 64.9 mL/min
- Injection volume: 2.0 µL

5.5.13.3 Detector.

- Temperature: 320 °C
- Hydrogen flow: 3.0. mL/min
- Air flow: 60 mL/min
- Mode: Constant column + makeup flow
- Combined flow: 10.0 mL/min
- Injection volume: 2.0 µL
- Makeup flow: On

5.5.13.4 Column: HP 5MS 25 m x 0.25 mm x 0.25 µm.

5.5.14 GC/MSD parameters. Instrument conditions may be changed to permit improved performance.

5.5.14.1 Acquisition Mode: Scan (50 – 550 amu)

5.5.14.2 Column: HP 5MS 25 m x 0.25 mm x 0.25 µm

5.5.14.3 Detector Temperature: 280 °C

5.5.14.4 Basic drug screen.

5.5.14.4.1 Oven Program

- Equilibration time: 0.50 minutes
- Initial temp: 110 °C
- Initial time: 1 minutes
- Ramp: 10 °C/min
- Final Temp: 290 °C
- Final Time: 9 minutes
- Run Time: 28 minutes

5.5.14.4.2 Inlet

- Mode: Splitless
- Temperature: 270 °C
- Injection volume: 1.0 µL
- Purge Time: ON at 1.0 minute

5.5.14.5 Acidic/neutral drug screen.

5.5.14.5.1 Oven Program

- Equilibration time: 0.50 minutes
- Initial temp: 120 °C
- Initial time: 0 minutes
- Ramp 1: 10 °C/min
- Final Temp 1: 260 °C
- Final Time 1: 0 minutes
- Ramp 2: 30 °C/min
- Final Temp 2: 300 °C
- Final Time 2: 2.67 minutes
- Run Time: 18 minutes

5.5.14.5.2 Inlet

- Mode: Splitless
- Temperature: 270 °C
- Injection volume: 1.0 µL
- Purge Time: ON at 1.0 minute

5.6 Procedure

5.6.1 **Extraction Option 1, Varian SPE Columns**

5.6.1.1 Label 16 x 125 mm screw cap tubes accordingly.

5.6.1.2 Pipet 1-3 mL of corresponding negative and positive control bloods and case sample bloods, fluids or tissue homogenates in appropriately labeled tubes.

5.6.1.3 Pipet internal standard into all tubes and vortex.

5.6.1.4 Add 9 mL of acetonitrile, cap and immediately shake each tube. Put tubes on mechanical rotator for 10 minutes. Note: urine samples do not require acetonitrile precipitation of proteins (start at step 5.6.1.8).

5.6.1.5 Centrifuge tubes for approximately 5 minutes at 2500 rpm to achieve separation.

5.6.1.6 Decant acetonitrile supernatant into labeled, disposable, 16 x 125 mm borosilicate glass culture tubes.

5.6.1.7 Evaporate acetonitrile to 1-2 mL in an evaporator/concentrator.

5.6.1.8 Add dH_2O to each tube to bring the total volume to approximately 3 mL

5.6.1.9 Add 2 mL of pH 6.0, 0.1 M potassium phosphate buffer to all tubes and vortex.

5.6.1.10 Solid phase extraction (SPE) Place labeled SPE cartridges in the extraction manifold. Throughout the SPE procedure, it is important not to permit the SPE sorbent bed to dry, unless specified. If necessary, add additional solvent/buffer to re-wet.

5.6.1.11 Condition columns with 2 mL methanol and aspirate.

5.6.1.12 Add 2 mL pH 6.0 phosphate buffer and aspirate.

5.6.1.13 Pour specimens into appropriate SPE columns. Aspirate slowly so that the sample takes at least 2 minutes to pass through the column.

5.6.1.14 Add 1 mL of 1 M acetic acid to each column and aspirate. Dry columns under full vacuum/pressure for at least 5 minutes.

5.6.1.15 If only extracting basic drugs, add 6 mL methanol, aspirate under full vacuum/pressure for at least 2 minutes and skip to step 5.6.1.21.

5.6.1.16 If extracting acidic/neutral and basic drugs, add 50 µL of methanol to each column and aspirate. Dry columns under full vacuum/pressure for at least 2 minutes.

5.6.1.17 Wipe the SPE column tips with Kimwipes®. Place labeled 10 mL conical test tubes in the manifold test tube rack. Be sure SPE column tips are in the designated conical tube.

5.6.1.18 Elute acid/neutral drugs by adding 2 mL of methanol to each column. Collect eluate in conical test tubes by gentle column aspiration or gravity drain.

5.6.1.19 Remove acid/neutral conical test tubes. Add an additional 4 mL methanol to all SPE columns and aspirate to waste under full vacuum/pressure.

5.6.1.20 Wipe the SPE column tips with Kimwipes®. Place labeled 10 mL conical test tubes in the manifold test tube rack. Be sure SPE column tips are in the designated conical tube.

5.6.1.21 Elute base drugs by adding 2 mL of ammonium hydroxide:ethyl acetate(2:98) to each column. Collect eluate in conical test tubes by column aspiration or gravity drain.

5.6.1.22 Evaporate eluates to dryness.

5.6.1.23 Add 100 µL ethyl acetate to the acid/neutral drug extracts. Vortex and transfer to autosampler vials.

5.6.1.24 Add 50 µL ethyl acetate to basic drug extracts. Vortex and transfer to autosampler vials.

5.6.1.25 Transfer autosampler vials to the GC-NPD and/or GCMS. Extracts with drugs indicated by retention time on NPD are re-injected on GCMS for confirmation. Alternately, extracts may be injected directly on the GCMS without NPD analysis. Drug retention time and GCMS spectral match are used to identify drugs.

5.6.2 **Extraction Option 2, CleanScreen SPE Columns.**

5.6.2.1 Label clean 16 x 125 mm screw cap tubes accordingly.

5.6.2.2 Pipet 1-3 mL of corresponding negative and positive control bloods and case sample bloods, fluids or tissue homogenates in appropriately labeled tubes.

5.6.2.3 Pipet internal standard into all tubes and vortex.

5.6.2.4 Add 6.0 mL deionized water to each tube. Mix, vortex briefly and let stand for 5 minutes.

5.6.2.5 Centrifuge at approx 2000 rpm for 10 minutes. Transfer supernatant to clean 16 x 125 mm tubes and discard the tube with the remaining pellet.

5.6.2.6 Add 3.0 mL of pH 6 phosphate buffer, mix and vortex. As necessary adjust the pH to 5.5 to 6.5 with additional 0.1 M phosphate buffer.

5.6.2.7 Solid phase extraction. Throughout the SPE procedure, it is important not to permit the SPE sorbent bed to dry, unless specified. If necessary, add additional solvent/buffer to re-wet.

5.6.2.8 Add 3 mL hexane to each column and aspirate on vacuum manifold

5.6.2.9 Add 3 mL methanol to each column and aspirate on vacuum manifold.

5.6.2.10 Add 3 mL dH_2O and aspirate.

5.6.2.11 Add 1 mL of 0.1 M pH 6.0 phosphate buffer and aspirate

5.6.2.12 Without delay, pour specimens into appropriate SPE columns. Elute from cartridges under vacuum at approximately 1-2 mL/ minute flow.

5.6.2.13 Add 3 mL dH_2O and aspirate at ≤ 3inches of mercury.

5.6.2.14 Repeat the dH_2O wash a second time.

5.6.2.15 Wash with 2.0 mL 1.0 M acetic acid and aspirate.

5.6.2.16 If only extracting basic drugs, add 3 mL methanol, aspirate under full vacuum/pressure for at least 2 minutes and skip to step 5.6.2.24.

5.6.2.17 If extracting acidic/neutral and basic drugs, dry columns under full vacuum/pressure for at least 2 minutes.

5.6.2.18 Add 2 mL hexane and aspirate.

5.6.2.19 Wipe the SPE column tips with Kimwipes®. Place labeled 10 mL conical test tubes in the manifold test tube rack. Be sure SPE column tips are in the designated conical tube.

5.6.2.20 Elute acid/neutral drugs by adding 3 mL of hexane/ethyl acetate (50:50 v/v) to each column. Collect eluate in conical test tubes by gentle column aspiration or gravity drain.

5.6.2.21 Remove acid/neutral conical test tubes. Add an additional 3 mL methanol to all SPE columns and aspirate to waste under full vacuum/pressure.

5.6.2.22 Add 2 mL hexane to each column. Dry columns at ≥ 10 inches of mercury for five minutes.

5.6.2.23 Wipe the SPE column tips with Kimwipes®. Place labeled 10 mL conical test tubes in the manifold test tube rack. Be sure SPE column tips are in the designated conical tube.

5.6.2.24 Elute basic drugs by adding 3 mL of freshly prepared methylene chloride/isopropanol/ammonium hydroxide solution to each column. Collect eluate in conical test tubes by column aspiration or gravity drain.

5.6.2.25 Elute at 1 - 2 mL/minute (no vacuum) and collect eluates.

5.6.2.26 Evaporate eluates at < 60° C just to dryness.

5.6.2.27 Reconstitute the residue with 50 µL of toluene/ hexane/isoamyl alcohol

5.6.2.28 Vortex and transfer to autosampler vials.

5.6.2.29 Transfer autosampler vials to the GC-NPD and/or GCMS. Extracts with drugs indicated by retention time on NPD are re-injected on GCMS for confirmation. Alternately, extracts may be injected directly on the GCMS without NPD analysis. Drug retention time and GCMS spectral match are used to identify drugs.

5.6.3 **Extraction Option 3, Basic LLE**

5.6.3.1 Label clean 16 x 125 mm screw cap tubes accordingly.

5.6.3.2 Pipet 1-3 mL of corresponding negative and positive control bloods and case sample bloods, fluids or tissue homogenates in appropriately labeled tubes.

5.6.3.3 Pipet internal standard into all tubes and vortex.

5.6.3.4 Add 2 mL of saturated borate buffer to each tube.

5.6.3.5 Add 5 mL of toluene/ hexane/isoamyl alcohol extraction solvent to each tube.

5.6.3.6 Rotate tubes for 20 minutes.

5.6.3.7 Centrifuge at approx 2000 rpm for 15 minutes.

5.6.3.8 Transfer the top (organic) layer to appropriately labeled 13 x 100 mm screw-cap test tubes. Discard lower (aqueous) layer.

5.6.3.9 Add 2 mL of 0.5 N sulfuric acid to tubes. Cap and rotate 20 minutes. Centrifuge at approx 2000 rpm for 15 minutes.

5.6.3.10 Aspirate off top (organic) layer and discard.

5.6.3.11 Adjust aqueous layer to a basic pH by slowly adding solid 3:2 $NaHCO_3/K_2CO_3$ buffer until effervescence ceases. Then add approximately 0.3 g excess $NaHCO_3/K_2CO_3$ buffer to saturate the aqueous layer.

5.6.3.12 Add 200 µL of toluene/ hexane/isoamyl alcohol extraction solvent to each tube, cap tubes and vortex for 10-15 seconds. Centrifuge tubes for 5 minutes.

5.6.3.13 Transfer approximately 200 µL of top (organic) layer into GC autosampler vials.

5.6.3.14 Transfer autosampler vials to the GC-NPD and/or GCMS. Extracts with drugs indicated by retention time on NPD are re-injected on GCMS for confirmation. Alternately, extracts may be injected directly on the GCMS without NPD analysis. Drug retention time and GCMS spectral match are used to identify drugs.

5.6.4 **Extraction Option 4, Acid/Neutral LLE**

5.6.4.1 Label clean 16 x 125 mm screw cap tubes accordingly.

5.6.4.2 Pipet 1-3 mL of corresponding negative and positive control bloods and case sample bloods, fluids or tissue homogenates in appropriately labeled tubes.

5.6.4.3 Pipet internal standard into all tubes and vortex.

5.6.4.4 Add 1 mL of pH 5.5 sodium phosphate buffer to each tube.

5.6.4.5 Add 5 mL of ethyl acetate to each tube.

5.6.4.6 Rotate tubes for 20 minutes.

5.6.4.7 Centrifuge at approx 2000 rpm for 15 minutes.

5.6.4.8 Transfer the top (organic) layer to appropriately labeled 13 x 100 mm screw-cap test tubes.

5.6.4.9 Evaporate to dryness at 50-60°C under nitrogen.

5.6.4.10 Reconstitute each sample with 0.5 mL hexane. Vortex briefly.

5.6.4.11 Add 2 mL 0.1 N HCl to each tube. Vortex for 30 seconds.

5.6.4.12 Centrifuge at approx 2000 rpm for 15 minutes.

5.6.4.13 Aspirate and discard upper (organic) layer.

5.6.4.14 Transfer bottom (aquesous) layer to appropriately labeled 13 x 100 mm screw-cap test tubes.

5.6.4.15 Add 5 mL chloroform to each tube. Vortex for 30 seconds.

5.6.4.16 Centrifuge at approx 2500 rpm for 10 minutes.

5.6.4.17 Aspirate and discard upper layer.

5.6.4.18 Tranfer bottom layer to appropriately labeled 13 x 100 mm screw-cap test tubes.

5.6.4.19 Evaporate to dryness at 50-60°C under nitrogen.

5.6.4.20 Reconstitute samples with 50 µL ethyl acetate.

5.6.4.21 Vortex briefly and transfer to autosampler vials.

5.6.4.22 Transfer autosampler vials to the GC-NPD and/or GCMS. Extracts with drugs indicated by retention time on NPD are re-injected on GCMS for confirmation. Alternately, extracts may be injected directly on the GCMS without NPD analysis. Drug retention time and GCMS spectral match are used to identify drugs.

5.7 Calculation

5.7.1 GC/NPD Data.

5.7.1.1 Evaluate positive control to ensure efficiency of extraction and proper operation of the GC/NPD.

5.7.1.2 By comparing GC NPD retention times to known retention times from retention time tables (i.e. caffeine, nicotine and cotinine) some cases may be determined to be “negative” for drugs. In cases with peaks indicating the presence of drugs other than caffeine, nicotine and cotinine, reinject the extracts with significant findings on the GCMS for confirmation. Often, the GC NPD results may be useful when attempting to confirm drugs by GCMS.

5.7.2 GC/MSD Data

5.7.2.1 Case samples. Take spectra of significant peaks on the TIC. Include spectra
5.7.2.2 and spectral library matches for identified drugs or suspect compounds (excluding non-reported drugs such as caffeine, nicotine and cotinine). Do not include spectrums of “junk” peaks (e.g. fatty acids, pthalates, hydrocarbons, etc.). If needed, use extracted ion profiles to look for drugs indicated by history or GC/NPD results that are not significant peaks on the TIC. Label identified drugs on the TIC.

5.7.2.3 In order to estimate a drug’s concentration during screening, a semi-quantitative one-point calculation can be performed using positive controls. Use the drug-to-internal standard ratio (peak area) of the case

sample and positive control ratio and concentration to calculate the case sample's drug concentration. Note: the same internal standard must be used for both mix and case sample.

5.7.2.3.1 Quantitation factor: Divide the drug of interest's area by the internal standard's area to obtain the drug-to-internal standard ratio. Divide the drug's concentration by the drug-to-internal standard ratio. The resulting quotient is the quantitation factor.

5.7.2.3.2 Case Sample. Divide the drug of interest's area by the internal standard's area to obtain the drug-to-internal standard ratio. Multiple this ratio by the appropriate quantitation factor to obtain the approximate drug concentration of drug in the case sample. Semi-quantitative concentrations are used to plan dilutions or expected concentrations for additional drug quantitations and to determine whether performing a drug quantitation is necessary or toxicologically significant.

5.7.3 Quantitation. Prepare a response curve of area (height) of analyte to area (height) of internal standard ratio versus calibrator concentration. Calculate the analyte concentration by interpolation of the linear plot.

5.7.4 Negative Control. The negative control is used as an interpretative aid in assessing internal standard recovery and identifying "junk" peaks that may be common in all samples.

5.8 Quality Control And Reporting

5.8.1 See Toxicology Quality Guidelines SOP for quality control and reporting.

5.9 References

5.9.1 Varian Bond Elute Certify™ Instruction Manual

5.9.2 T. Soriano, C. Jurado, M. Menendez and M. Repetto, "Improved Solid-Phase Extraction Method for Systematic Toxicological Analysis in Biological Fluids," J. Anal. Toxicol. 2001; March (25): 137-143.

5.9.3 W.H. Anderson and D.C. Fuller, "A Simplified Procedure for the Isolation, Characterization, and Identification of Weak Acid and Neutral Drugs from Whole Blood," J. Anal. Toxicol. 1987, Sep/Oct (11): 198-204.

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